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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/226,794	01/07/99	DEBINSKI	W 6460-4

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EXAMINER	
UNGAR, S	
ART UNIT	**PAPER NUMBER**
1642	20

DATE MAILED: 11/06/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 09/226,794	Applicant(s) Debrinski et al
	Examiner Ungar	Art Unit 1642

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *three* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on *Aug 2, 2001* .

2a) ☐ This action is **FINAL.** 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) *1, 2, 4-6, and 14-22* is/are pending in the application.

4a) Of the above, claim(s) _____ is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) *1, 2, 4-6, and 14-22* is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved.

12) ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

15) ☒ Notice of References Cited (PTO-892)

16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

17) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____

18) ☑ Interview Summary (PTO-413) Paper No(s). 19

19) ☐ Notice of Informal Patent Application (PTO-152)

20) ☐ Other:

1. Upon review and reconsideration and in view of the previous request for suspension of action under 37 CFR 1.103(b), the finality of the previous Office Action is withdrawn.

2. The Amendments filed July 16, 2001 (Paper No. 17) and August 2, 2001 (Paper No. 18) and the Declaration filed July 16, 2001 (Paper No. 16) in response to the Office Actions of May 28, 2001 (Paper No. 15) and November 13, 2000 (Paper No. 12) are acknowledged. Paper No 17 has not been entered as the amendments were not properly submitted. Paper Nos 16 and 18 have been entered. Previously pending claims 1, 2, 4 and 5 have been amended and new claims 14-22 have been added. Claims 1, 2, 4-6 and 14-22 are currently being examined.

3. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

New Grounds of Rejection

Claim Rejections - 35 USC § 103

4 Claims 1, 2, 4-6 and 14-22 are rejected under 35 USC 103 as being unpatentable over US Patent No. 5,614,191, of record in view of Debinski et al (JBC, 1996, 271:22428-22433, see attached), of record essentially for the reasons previously set forth in Paper No. 9, Section 12, pages 8-10 and reiterated in Paper No. 12, Section 8, pages 6-7 and further in view of BioCentury Extra (1996, 465:1) as evidenced by Debinski et al, Abstract, 17th International Congress, 1998, Rio de Janeiro, Brazil, IDS item.

In the interests of compact prosecution, it is assumed for examination purposes that the hIL-13 receptor specifically and preferentially binds hIL13 compared to hIL4.

The claims are drawn to a method of reducing the rate of growth of glioma cells *in vivo* in a mammalian subject, the glioma cells comprising an IL13-specific receptor that specifically binds IL13 but not IL4 comprising delivering into the subject a molecule having an IL13-moiety and a cytotoxic moiety in an amount effective to reduce the rate of growth of glioma cells (claim 1), wherein the glioma cells are glioblastoma multiforme cells (claim 2), wherein the cells form a tumor in the subject and the growth of the tumor is inhibited (claim 4), wherein the tumor volume is reduced (claim 5), wherein the molecule is delivered by intratumoral injection (claim 6), wherein the tumor is located in the cranium of the mammalian subject (claim 14), wherein the IL13 moiety is hIL-13 (Claim 15), wherein the cytotoxic moiety is Diphtheria toxin (16), Pseudomonas toxin (claim 17), a method of killing a glioma cell in situ essentially comprising the steps of claim 1 (claim 18), wherein the glioma cell is a multiforme glioblastoma multiforme cell (claim 19) wherein the IL13 moiety is hIL13 (claim 20), wherein the cytotoxic moiety is a Diphtheria toxin (claim 21), Pseudomonas toxin (claim 22).

US Patent No. 5,614,191 and Debinski et al teach as set forth previously. US Patent No.5,614,191 further teaches that preferred cytotoxic effector molecules for use in the method include Pseudomonas exotoxin and Diphtheria toxin (col 2, lines 42-44). Debinski et al further teach that patients with relapsed brain tumors in particular appear to be very responsive to chimeric toxins and that in particular,

glioma cells are extremely sensitive to hIL13-PE38QQR chimeric toxin and that receptors for hIL13 are significantly overexpressed on primary human glioblastoma multiforme cells and that one predominant form of IL13R is found on primary human glioblastoma multiforme cells (p. 22428, col 2) which is sensitive to hIL13-PE chimeric toxin which was not inhibited by hIL4 (see Fig. 1, p. 22429) wherein the cells do not interact with hIL4 (p. 22432, col 1). This suggests that the hIL13 on glioma cells is a cancer-specific receptor for hIL13 (p.22433, col 1). The explant glioblastoma multiforme glioma cells have 300,000-500,000 hIL13 binding sites per cell and are more enriched in hIL13 receptors than established glioma cell cultures (p. 22431, col 1). These hIL13 receptors represent a new attractive target for the treatment of brain cancers (p. 22433, col 1). Given the findings of Debinski et al, Abstract, 17th International Cancer Congress, it is an inherent property of the receptors to be overexpressed in situ on GBM since Debinski specifically shows that the receptors are overexpressed in primary slice samples of GBM. The combined references teach as set forth previously and above, but do not teach the tumor is located in the cranium, do not teach intratumor administration of the molecule,

BioCentury Extra teaches that glioblastoma multiform is a common form of brain cancer (see abstract).

Debinski et al, Abstract, 17th International Congress teach autoradiographic studies that demonstrate directly the binding of hIL13 to malignant brain tissue which confirm the original observation that hIL13R which binds hIL13 but does not share that binding with hIL4 is overexpressed in human glioma and that the

receptor constitutes a new, unique target for delivery of cytotoxic therapies for GBM.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use the method of US Patent No. 5,614,191 to treat a human patient with human glioblastoma multiforme tumor cells, wherein the receptor binds hIL13 but not hIL4, because Debinski et al, 1996, teach that primary human glioblastoma multiforme cells overexpress hIL13R which binds hIL13 but does not interact with hIL4 and are extremely sensitive to a chimeric protein (composed of hIL13 and PE38QQR which appears to be the same construct used in the method of US Patent No. 5,614,191) and because US Patent No.5,614,191 specifically claims a method of impairing growth of a solid tumor cell bearing an IL-13 receptor. One of ordinary skill in the art would have been motivated to use, and would have expected to successfully use, the method of US Patent No. 5,614,191 to treat a human patient with human glioblastoma multiforme tumor cells, wherein the receptor binds hIL13 but not hIL4, because US Patent No. 5,6615,191 specifically claims a method of impairing growth of a solid tumor cell bearing an IL-13 receptor and because Debinski et al, 1996 have clearly taught that human glioblastoma multiforme cells are sensitive to and bind the claimed construct.

Further, it would have been *prima facie* obvious to, and one of ordinary skill in the art at the time the invention was made would have been motivated to treat the tumor in the cranium, in situ by intratumoral injection because BioCentury Extra teaches that glioblastoma multiform is a common form of brain cancer and the brain

is located in the cranium and because intratumoral injection of therapeutics was conventional in the art at the time the invention was made. One of ordinary skill in the art would have been motivated to intratumorally/in situ inject the molecule in order to avoid the problems associated with, for example, intravenous administration, which results in not only the dilution of the therapeutic but also renders it highly vulnerable to attack by the immune system.

Further, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, and one would have been motivated to, substitute a Diphtheria toxin for the Pseudomonas toxin in the construct of US Patent No.5,614,191 because US Patent No.5,614,191 specifically teaches that the equivalence of the two toxins for use in the method.

Applicant's arguments drawn to the rejection of claims 1-5 under 35 USC 103 are relevant to the instant rejection.

Applicant argues that the sensitivity assay drawn to explant cells are *in vitro* assays which were not performed in situ and that there are differences between *in vitro* and *in situ* assays, in particular, glioma cells that express an antigen *in situ* lose expression of that antigen when explanted and cultured *in vitro* and that overexpression of a molecule observed *in vitro* cell cultures did not occur *in situ* situation. Since there are differences between *in vitro* cell cultures and cells located *in situ*, the Debinski Paper in combination with the '191 patent, does not render the invention obvious.

The argument has been considered but has not been found persuasive because it is clear from Debinski et al, 1996, that the explant cells do not lose expression of

the hIL13R antigen and that the antigen is expressed at a concentration ten times more than that found in the cell lines (see p. 22433, col 1, last paragraph). Contrary to Applicant's arguments, given the extensive overexpression of the receptor in the tumor explant, it would be expected that the antigen would be extensively overexpressed *in situ* and one would have expected to successfully treat a mammal with the method of the combined references. Finally, given the Debinski et al 1998 findings, it is clear that overexpression of the receptor *in situ* is an inherent property of the receptor on GBM.

5. Claims 1, 2, 4-6 and 14-22 are rejected under 35 USC 103 as being unpatentable over US Patent No. 5,614,191, of record in view of Debinski et al (JBC, 1996, 271:22428-22433), of record essentially for the reasons previously set forth in Paper No. 9, Section 12, pages 8-10 and reiterated in Paper No. 12, Section 8, pages 6-7 and further in view of BioCentury Extra (1996, 465:1).and Debinski et al, Abstract, 17th International Congress, 1998, Rio de Janeiro, Brazil, IDS item.

In the interests of compact prosecution, it is assumed for examination purposes that the hIL-13 receptor specifically and preferentially binds hIL13 compared to IL4.

The claims are drawn to a method of reducing the rate of growth of glioma cells *in vivo* in a mammalian subject, the glioma cells comprising an IL13-specific receptor that specifically binds IL13 but not Il4 comprising delivering into the subject a molecule having an IL13-moiety and a cytotoxic moiety in an amount effective to reduce the rate of growth of glioma cells (claim 1), wherein the glioma cells are glioblastoma multiforme cells (claim 2), wherein the cells form a tumor in

the subject and the growth of the tumor is inhibited (claim 4), wherein the tumor volume is reduced (claim 5), wherein the molecule is delivered by intratumoral injection (claim 6), wherein the tumor is located in the cranium of the mammalian subject (claim 14), wherein the IL13 moiety is hIL-13 (Claim 15), wherein the cytotoxic moiety is Diphtheria toxin (16), Pseudomonas toxin (claim 17), a method of killing a glioma cell in situ essentially comprising the steps of claim 1 (claim 18), wherein the glioma cell is a multiforme glioblastoma multiforme cell (claim 19) wherein the IL13 moiety is hIL13 (claim 20), wherein the cytotoxic moiety is a Diphtheria toxin (claim 21), Pseudomonas toxin (claim 22).

US Patent No.5,614,191 and Debinski et al teach as set forth previously. US Patent No.5,614,191 further teaches that preferred cytotoxic effector molecules for use in the method include Pseudomonas exotoxin and Diphtheria toxin (col 2, lines 42-44). Debinski et al further teach that patients with relapsed brain tumors in particular appear to be very responsive to chimeric toxins and that in particular, glioma cells are extremely sensitive to hIL13-PE38QQR chimeric toxin and that receptors for hIL13 are significantly overexpressed on primary human glioblastoma multiforme cells and that one predominant form of IL13R is found on primary human glioblastoma multiforme cells (p. 22428, col 2) which is sensitive to hIL13-PE chimeric toxin which was not inhibited by hIL4 (see Fig. 1, p. 22429) wherein the cells do not interact with hIL4 (p. 22432, col 1). This suggests that the hIL13 on glioma cells is a cancer-specific receptor for hIL13 (p.22433, col 1). The explant glioblastoma multiforme glioma cells have 300,000-500,000 hIL13 binding sites per cell and are more enriched in hIL13 receptors than established glioma cell

cultures (p. 22431, col 1). These hIL13 receptors represent a new attractive target for the treatment of brain cancers (p. 22433, col 1). The combined references teach as set forth previously and above, but do not teach the tumor is located in the cranium, do not teach intratumor administration of the molecule.

Debinski et al, 1998 teach autoradiographic studies that demonstrate directly the binding of hIL13 to malignant brain tissue which confirm the original observation that hIL13R which binds hIL13 but does not share that binding with hIL4 is overexpressed in human glioma and that the receptor constitutes a new, unique target for delivery of cytotoxic therapies for GBM.

BioCentury Extra teaches that glioblastoma multiform is a common form of brain cancer (see abstract).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use the method of US Patent No. 5,614,191 to treat a human patient with human glioblastoma multiforme tumor cells, wherein the receptor binds hIL13 but not hIL4, because Debinski et al, 1996, teach that primary human glioblastoma multiforme cells overexpress hIL13R which binds hIL13 but does not interact with hIL4 and are extremely sensitive to a chimeric protein (composed of hIL13 and PE38QQR which appears to be the same construct used in the method of US Patent No. 5,615,191) and because Debinski et al, 1998, 17th International Cancer Congress specifically teach that the receptor is overexpressed on primary slice samples of GBM and because US Patent No. 5,614,191 specifically claims a method of impairing growth of a solid tumor cell bearing an IL-13 receptor. One of ordinary skill in the art would have been motivated to use,

and would have expected to successfully use, the method of US Patent No. 5,615,191 to treat a human patient with human glioblastoma multiforme tumor cells, wherein the receptor binds hIL13 but not hIL4, because US Patent No. 5,6615,191 specifically claims a method of impairing growth of a solid tumor cell bearing an IL-13 receptor and because Debinski et al, 1996 and 1998, 17th International Cancer Congress have clearly taught that not only explant tumor cells but also uncultured primary tumor cells overexpress the receptor which would be expected to be sensitive to and bind the claimed construct and further because Debinski et al, 1998, 17th International Cancer Congress have clearly taught that the receptor constitutes a new and unique target for delivery of cytotoxic therapies.

Further, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to treat the tumor in the cranium, in situ by intratumoral injection because BioCentury Extra teaches that glioblastoma multiform is a common form of brain cancer and the brain is located in the cranium and because intratumoral injection of therapeutics was conventional in the art at the time the invention was made. One of ordinary skill in the art would have been motivated to intratumorally/in situ inject the molecule in order to avoid the problems associated with, for example, intravenous administration, which results in not only the dilution of the therapeutic but also renders it highly vulnerable to attack by the immune system.

Further, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made, and one would have been motivated to, substitute a Diphtheria toxin for the Pseudomonas toxin in the construct of US

Patent No. 5,614,191 because US Patent No. 5,614,191 specifically teaches that the equivalence of the two toxins for use in the method.

Claim Rejections - 35 USC § 112

6. Claims 1, 2, 4-6 and 14-22 are rejected under 35 USC 112, first paragraph, as the specification does not contain a written description of the claimed invention. The limitation of a receptor that binds IL13 but not IL4 has no clear support in the specification and the claims as originally filed. A review of the specification reveals support for the presence of a tumor bearing an IL-13-specific receptor (p. 3, lines 15-17), IL13-specific receptor is expressed by tumor cells other than malignant glioma cells (p. 4, lines 20-32), and methods of inhibiting the growth of tumors bearing an IL13-specific receptor (p. 5, lines 1-4), both IL13 and IL4 bind to a functional IL13/IL4 receptor that is overexpressed on some adenocarcinomas (p. 6, lines 1-5), the definition of an "IL13-specific receptor" as used herein is a receptor that binds to IL13 to a much greater extent than it binds IL4 (p. 6, lines 10-13). There is no mention of a receptor that specifically binds IL13 but not IL4. Given the definition of an IL13-specific receptor, it is clear that both IL13 and IL4 bind specifically to an IL13-specific receptor but that the affinity of IL13 is greater than the affinity of IL4. The subject matter claimed in claims 1, 2, 4,-6 and 14-22 broadens the scope of the invention as originally disclosed in the specification.

7. No claims allowed.

8. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Susan Ungar, PhD whose telephone number is

(703) 305-2181. The examiner can normally be reached on Monday through Friday from 7:30am to 4pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony Caputa, can be reached at (703) 308-3995. The fax phone number for this Art Unit is (703) 308-4242.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Effective, February 7, 1998, the Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 1642.

Susan Ungar
Primary Patent Examiner
October 30, 2001

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Receptor for Interleukin (IL) 13 Does Not Interact with IL4 but Receptor for IL4 Interacts with IL13 on Human Glioma Cells*

(Received for publication, March 11, 1996, and in revised form, June 10, 1996)

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Recently, we have demonstrated that human (h) glioma cell lines express large number of receptors (R) for interleukin 13 (IL13) (Debinski, W., Obiri, N. I., Powers, S. K., Pastan, I., and Puri, R. K. (1995) *Clin. Cancer Res.* 1, 1253–1258). These cells are extremely sensitive to a chimeric protein composed of hIL13 and a derivative of *Pseudomonas* exotoxin (PE), PE38QQR. We have found that the cytotoxicity of hIL13-PE38QQR was blocked by hIL13 but not by hIL4 on the U-251 MG and U-373 MG cells, contrary to what was observed on several adenocarcinoma cell lines. In the present study, we further explored interactions between receptor for IL13 and IL4 on glioma cells. Established human glioma cell lines, such as DBTRG MG, Hs 683, U-87 MG, SNB-19, and A-172, are very susceptible to hIL13-PE38QQR, and the action of the chimeric toxin is not blocked by hIL4 on all these cells either. Also, hIL4 is not a competitor for ^{125}I-hIL13 binding sites on glioma cells. Of interest, a corresponding hIL4-based chimeric toxin, hIL4-PE38QQR, is poorly active or not active on all the tested glioma cell lines. When active, however, hIL4 toxin action was blocked by hIL13. hIL13 is a competitor for ^{125}I-hIL14 binding in a competitive binding assay on glioma cells. hIL13 and hIL4 did not affect the growth of the tested glioma cell lines. Human glioblastoma multiforme explant cells exhibited similar responses to the chimeric toxins and interleukins when compared with that found in established glioma cultures. Our results suggest that the hIL13R on glioma cells is expressed in one predominant form, the form that does not interact with IL4. Thus, this type of hIL13R is apparently different from the one demonstrated previously on several adenocarcinoma cell lines.

Chimeric toxic fusion proteins composed of targeting ligands and bacterial toxins, such as *Pseudomonas* exotoxin (PE),[1] are rationally designed promising compounds to be used in cancer treatment (1). Such chimeric toxins are in the initial steps of preclinical and clinical evaluation, and prominent antitumor activities were observed (2–4). The patients with relapsed brain tumors in particular appear to be very responsive to chimeric toxins (3). PE is a multidomain/multifunctional globular protein of M_r 66,000 (5, 6). Domain Ia of PE is the α_2-macroglobulin receptor binding domain (7). Domain II must be cleaved by furin (8) in order to activate the toxin and enable the active portion of the toxin to translocate into the cytosol (9). Domain III of PE contains the ADP-ribosylation activity (10). This domain inactivates elongation factor 2 that leads to cell death.

Cancer cells of solid tumor origins express receptors for interleukins, such as interleukin 4 (IL4), a hemopoietic growth factor, and these receptors constitute an attractive target for anticancer therapy using chimeric toxins (4, 11–13). We have recently shown the presence of a common internalized receptor for hIL4 and hIL13 on a series of human adenocarcinoma cell lines (14). These cell lines are killed potently by chimeric toxins composed of hIL13 or hIL4 and a derivative of PE, PE38QQR. hIL13 and hIL4 blocked reciprocally the cytotoxicities of their respective chimeric toxins. We hypothesized that the common receptor for the two cytokines on the studied adenocarcinoma cells is composed of the main 140-kDa subunit of the hIL4R (15, 16) and a 70-kDa hIL13-binding subunit (17). This is because (i) a chimeric toxin must be internalized in a process of receptor-mediated endocytosis in order to allow a proper toxin processing and intracellular routing that results in cell death (1), and (ii) the 140-kDa hIL4R has been shown to be the subunit of hIL4R that internalizes in response to hIL4 binding (16). More recently, we have found a significant overexpression of hIL13R on human glioma cells, and the glioma cells are extremely sensitive to hIL13-PE38QQR (18). Of interest, hIL4 did not block the action of hIL13-PE38QQR on the first two tested glioma cell lines unlike on solid tumor cell lines of peripheral origins (14, 18). These findings raised a possibility of the presence of hIL13R that does not interact with IL4 and is overexpressed on some glioma cells. On the other hand, several recent reports suggested a pattern for IL13- and IL4R commonality in which cells that do bind IL13 should bind IL4, and IL13 binding is always fully competed for by IL4 (19–21).

In this study, we have further examined interactions between hIL13R and hIL4R in human glioma cells. We used five more established human glioma cell lines and, for the first time, cells of a human glioblastoma multiforme explant, and tested the cytotoxicities of chimeric toxins and responses to hIL13 and hIL4. Our results indicate that one predominant form of hIL13R is overexpressed on glioma cells. These results should be helpful in understanding the biology of the interleukin receptors and may be important in designing therapeutic strategies that target them.

* This work was supported by the Surgery Feasibility Research Grant (to W. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence and requests for reprints should be addressed: Division of Neurosurgery, Dept. of Surgery, The Milton S. Hershey Medical Center, Pennsylvania State University, 500 University Dr., Hershey, PA 17033-0850. Tel.: 717-531-4541; Fax: 717-531-3858; E-mail: debinski@debin.nsr.hmc.PSU.edu.

[1] The abbreviations used are: PE, *Pseudomonas* exotoxin A; R, receptor; h, human; r, recombinant; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt; PMS, phenazine methasulfate.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases and DNA ligase were obtained from New England Biolabs (Beverly, MA), Life Technologies, Inc., and Boehringer Mannheim. [^{3}H]Leucine and ^{125}I were purchased from Amersham Corp. Fast protein liquid chromatography columns, media, and Ficoll-Paque were purchased from Pharmacia Biotech Inc. Oligonucleotide primers were synthesized at Pharmacia's Gene Assembler at the Research Center, HDM-UM. Polymerase chain reaction kit was from Perkin-Elmer. MTS/PMS (see below) for cell titer 96 aqueous nonradioactive cell proliferation assay was purchased from Promega (Madison, WI).

Plasmids, Bacterial Strains and Cell Lines—Plasmids carry a T7 bacteriophage late promoter, a T7 transcription terminator at the end of the open reading frame of the protein, a f1 origin of replication, and gene for ampicillin resistance (22). The cDNA encoding sequence for hIL13 was polymerase chain reaction-cloned to produce hIL13-PE38QQR, as described previously (14). Recombinant proteins were expressed in *Escherichia coli* BL21 (λDE3) under control of the T7 late promoter (23). Plasmids were amplified in *E. coli* (HB101 or DH5α high efficiency transformation) (Life Technologies, Inc.) and DNA was extracted using Qiagen kits (Chatsworth, CA).

The cytotoxic activity of chimeric toxins and antiproliferative activity of ILs were tested on several brain tumor cell lines, such as U-373 MG, DBTRG-05 MG, A-172, Hs 683, U-251 MG, and SW-1088. The majority of cell lines were obtained from the ATCC, and they were maintained under conditions recommended by the ATCC. The SW-1088 cell line was a gift of Dr. J. Connor (Pennsylvania State University College of Medicine).

Glioma Explant Cells Preparation—Pathology-proven surgical specimen of glioblastoma multiforme was collected and transferred to the laboratory under sterile conditions. Peripheral and necrotic tissues were excised, and the remaining tissue was minced using a scalpel. Tumor tissue was incubated in a mixture composed of collagenase types II and IV, Dispase, and NuSerum/Dulbecco's modified Eagle's medium, at 37 °C with constant shaking for 45 min. Cell suspension was then passed through gauze and washed first with Hanks' balanced salt solution and then with phosphate-buffered saline (Ca^{2+}, Mg^{2+}-free). Cells were then layered on the Ficoll-Paque and centrifuged at 400 × *g* at 18–20 °C for 35 min. The isolated cells were resuspended in 3 × volume of balanced salt solution and centrifuged at 100 × *g* at 18–20 °C for 10 min. The pellet was washed one more time with the same solution and finally resuspended in RPMI 1640/25 mM HEPES with L-glutamine and supplemented with 10% fetal calf serum, 0.1 mg/ml L-cystine, 0.02 mg/ml L-proline, 0.1 mg/ml sodium pyruvate, HT supplement, and antibiotics. The cells were transferred into 100-mm plates and incubated at 37 °C in 95% O_2, 5% CO_2 humidified atmosphere.

Expression and Purification of Recombinant Proteins—*E. coli* BL21 (λDE3) cells were transformed with plasmids of interest and cultured in 1.0 liter of Terrific Broth. The chimeric toxins and interleukins were localized to the inclusion bodies. The procedure for the recombinant proteins isolation from the inclusion bodies was described previously (12). After dialysis, the renatured proteins were purified on ion-exchange columns and by size exclusion chromatography on Sephacryl S-200HR (Pharmacia).

Protein concentration was determined by the Bradford assay (Pierce "Plus") using bovine serum albumin as a standard.

Protein Synthesis Inhibition Assay—The cytotoxic activities of chimeric toxins, such as hIL13-PE38QQR and hIL4-PE38QQR, were tested as follows; usually 5 × 10^3 cells per well were plated in a 96-well tissue culture plate in 200 μl of media. Various concentrations of the chimeric toxins were diluted in 0.1% bovine serum albumin/phosphate-buffered saline, and 25 μl of each dilution was added to cells 20–28 h following cell plating. Cells were incubated at 37 °C for another 48 h. Then the cytotoxicity was determined using a colorimetric MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt)/PMS (phenazine methasulfate) cell proliferation assay. MTS/PMS was added at a half final concentration as recommended by the manufacturer. The cells were incubated with the dye for 6 h, and then the absorbance was measured at 490 nm for each well using a microplate reader (Cambridge Technology, Inc., Watertown, MA). The wells containing no cells, or wells with cells treated with high concentrations of PE or hIL13-PE38QQR (10 μg/ml), or wells with no viable cells left served as a background for the assay. For blocking studies, rhIL4 or rhIL13 was added to the cells for 60 min before the chimeric toxin addition. Data were obtained from the average of quadruplicate experiments, and the assays were repeated several times.

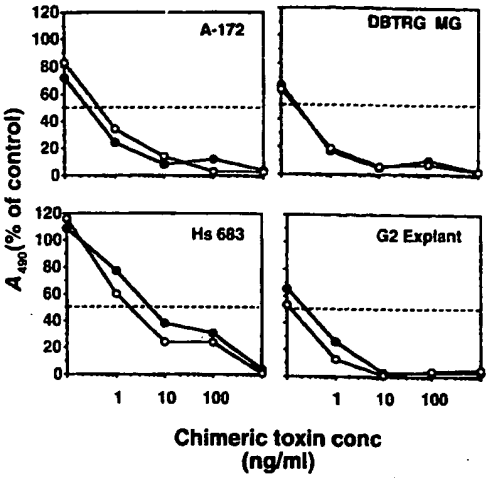

FIG. 1. **Cytotoxic activity of hIL13-PE38QQR on established glioma cell lines and human glioma (G2) explant cells and failure to inhibit this cytotoxicity by hIL4.** hIL4 was added at a concentration of 1.0 μg/ml. Three different batches of rhIL4 showed the same effect. The *dashed line* shows 50% of the difference between the background and control MTS conversion that was recorded at $A_{490\ nm}$. ●, hIL13-PE38QQR; ○, + hIL4.

To evaluate the effects of interleukins on cell proliferation, the assays were performed as follows. 1 × 10^3 cells per well were plated in a 96-well tissue culture plate in 200 μl of 0.5% fetal bovine serum-containing media, and the interleukins were added 20 h following cell plating. After 7- or 5-day incubation with the interleukins, MTS/PMS was added to the cells for 6 h, and the assay was performed as described above for the chimeric toxins.

Competitive Binding Assay—rhIL13 and rIL4 both produced in-house were labeled with ^{125}I (Amersham Corp.) by using the IODOGEN reagent (Pierce) according to the manufacturer's instructions. The specific activities of radiolabeled cytokines were estimated to be between 20 and 100 μCi/μg protein for ^{125}I-hIL13 and 179 μCi/μg protein for ^{125}I-hIL4.

Binding experiments were performed as described previously (17). Typically, 1 × 10^6 to 1.5 × 10^6 tumor cells were incubated at 4 °C for 2 h with ^{125}I-hIL13 (100–500 pM) or ^{125}I-hIL4 (100–500 pM) with or without increasing concentrations (up to 1000 nM) of unlabeled interleukins. The data were analyzed with the LIGAND program (17, 26) to determine receptor number and binding affinity.

RESULTS

To construct the chimeric toxin, the coding region of the IL13 gene was fused to a gene encoding a mutated form of PE, PE38QQR (14). PE38QQR has domain Ia and amino acids 365–380 in Ib deleted, plus the three lysine residues in domain III at positions 590, 606, and 613 are changed to two glutamines and arginine (QQR) (24). The chimeric gene is in the bacterial vector under the control of a bacteriophage T7 late promoter; the protein was expressed in *E. coli* BL21 (λDE3) as described previously (14). hIL4-PE38QQR (4), hIL4-PE4E (12), hIL4, and hIL13 were subcloned into the same expression plasmid and produced as was the hIL13-PE38QQR.

hIL13-PE38QQR Is Extremely Cytotoxic to Both Established Glioma Cell Lines and Glioblastoma Multiforme Explant Cells—We tested the A-172, DBTRG MG, and Hs-683 established human glioma cell lines and, for the first time, glioma explant cells (G2) to determine and/or confirm if hIL13-PE38QQR is cytotoxic to them. All the established glioma cell lines were very responsive with an IC_{50} (50% inhibitory concentration) of 0.1–5 ng/ml (Fig. 1). Of interest, human glioma explant cells were also extremely sensitive to the action of hIL13 toxin; the IC_{50} for hIL13-PE38QQR was 0.2 ng/ml (Fig. 1). The cytotoxic action of hIL13-PE38QQR was specific as it

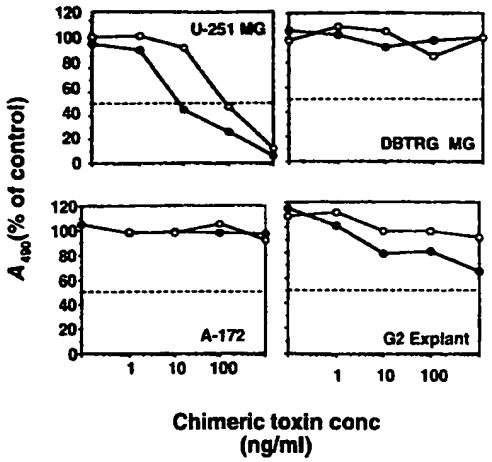

FIG. 2. **The cytotoxicity of hIL4-PE38QQR on glioma cells and blocking this cytotoxicity by hIL13.** hIL13 was added at a concentration of 1.0 μg/ml. The *dashed line* shows 50% of the difference between the background and control MTS conversion that was recorded at $A_{490\ nm}$. ●, hIL4-PE38QQR; ○, + hIL13.

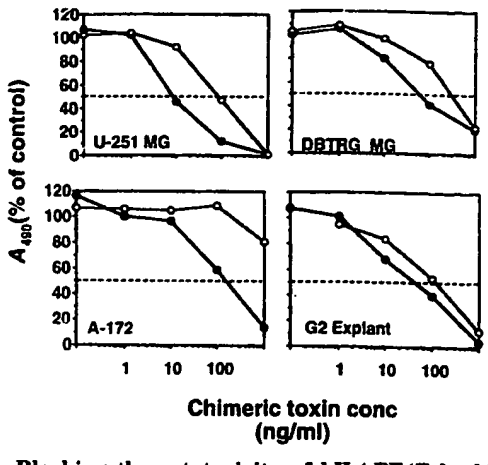

FIG. 3. **Blocking the cytotoxicity of hIL4-PE4E by hIL13 on glioma cells.** hIL13 was added at a concentration of 1.0 μg/ml. The *dashed line* shows 50% of the difference between the background and control MTS conversion that was recorded at $A_{490\ nm}$. ●, hIL4-PE4E; ○, +hIL13.

was blocked by an excess of hIL13 on all cells (18).[2] These data demonstrate that both established glioma cell lines and a primary culture of glioma cells possess hIL13 binding sites and such cells are extremely sensitive to hIL13-PE38QQR chimeric toxin.

hIL4 Does Not Block the Cytotoxicity of hIL13-PE38QQR on Glioma Cell Lines and Glioma Explant Cells—Because hIL13R has been shown to be related to IL4R (14, 17, 25), we explored further the specificity of hIL13-PE38QQR action on the glioma cell lines and G2 explant cells. The cells were treated with hIL13-PE38QQR with or without rhIL4 at a concentration of 1.0 μg/ml. The rhIL4 did not have any blocking action against hIL13-PE38QQR on either the established cultured cells (A-172, Hs 683, and DBTRG MG) or freshly explanted cultured glioma cells, even at a 1000-fold molar excess over the chimeric toxin (Fig. 1). These results indicate that the cell killing by the hIL13 toxin on these cells is independent of the presence of hIL4. The same results were obtained with the U-251 MG, U-373 MG (18), U-87 MG and SNB-19[2] cell lines.

Since these data are in contrast to observations made on several adenocarcinoma cells (14), we repeated the cytotoxicity experiments, for example, on Colo 201 human colon adenocarcinoma cells employing a colorimetric assay used in the present study (instead of tritium incorporation in Refs. 12, 14, and 18) and reproduced exactly the same results.[2]

hIL13 Blocks the Action of hIL4 Toxins on the U-251 MG, DBTRG MG, and A-172 Glioma Cells, and Glioma G2 Explant Cells—To investigate the possibility that hIL13 and IL4 may nevertheless compete, although not reciprocally, for the same binding site on glioma cells, we also treated the cells with hIL4-based recombinant toxin, hIL4-PE38QQR (4) (Fig. 2). It has previously been demonstrated that all tested glioma cell lines express specific 140-kDa hIL4R, as determined by a immunoreactivity of an antibody raised against the protein (13). We found, again unexpectedly, that hIL4-PE38QQR was without any significant specific cytotoxicity to most of these cells (Fig. 2) including the Hs-683 and U-373 MG cells.[2] Only the U-251 MG glioma cell line responded relatively well to hIL4-PE38QQR with an IC_{50} of 10 ng/ml. This cytotoxicity was blocked efficiently by an excess of hIL13 (Fig. 2). Thus, the cytotoxicity of hIL4-PE38QQR is blocked by an excess of hIL13; however, the cytotoxic action of hIL4-PE38QQR is absent on

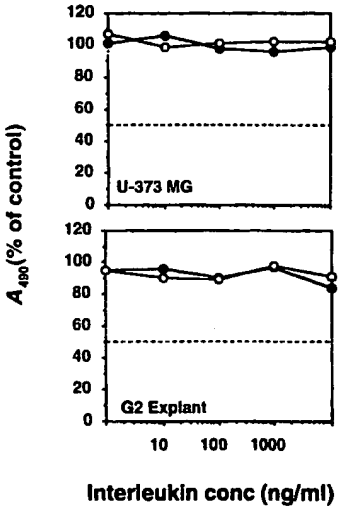

FIG. 4. **hIL13 and hIL4 do not inhibit proliferation of the U-373 MG and human glioma G2 explant cells.** Data represent in most cases the average of quadruplicate experiments. The *dashed line* shows 50% of the difference between the background and control MTS conversion that was recorded at $A_{490\ nm}$. ●, hIL13; ○, hIL4.

the majority of glioma cell lines and human glioma explant cells.

Since interleukins coupled to PE4E form of the toxin exhibit better cytotoxic activities on cancer cells (*e.g.* Ref. 12), we treated glioma cells also with hIL4-PE4E. Indeed, we have found higher cytotoxic potency of this chimeric protein when compared with hIL4-PE38QQR on several glioma cells as well as on G2 explant cells (Fig. 3). The IC_{50} ranged from 10 to 200 ng/ml on U-251 MG, DBTRG MG, A-172, G2 explant (Fig. 3), and U-87 MG[2] cells. The cytotoxic action of hIL4-PE4E was blocked by an excess of hIL13 (Fig. 3) in a similar fashion to blocking this cytotoxicity by hIL4.[2] The blocking on G2 cells was less than on other cell lines (Fig. 3), and a similar response was seen on SNB-19 cells.[2]

These results demonstrate that hIL4 and hIL13 have common binding sites on the glioma cell lines and are reminiscent of our previous findings on a series of adenocarcinoma cells (14). However, there is also a profound difference between our current and previous findings, since the commonality is not reciprocal, *i.e.*, only hIL13 is a competitor for the two receptors.

Antiproliferative Effects of hIL13 and hIL4 on Glioma

[2] W. Debinski, R. Miner, and R. K. Puri, unpublished results.

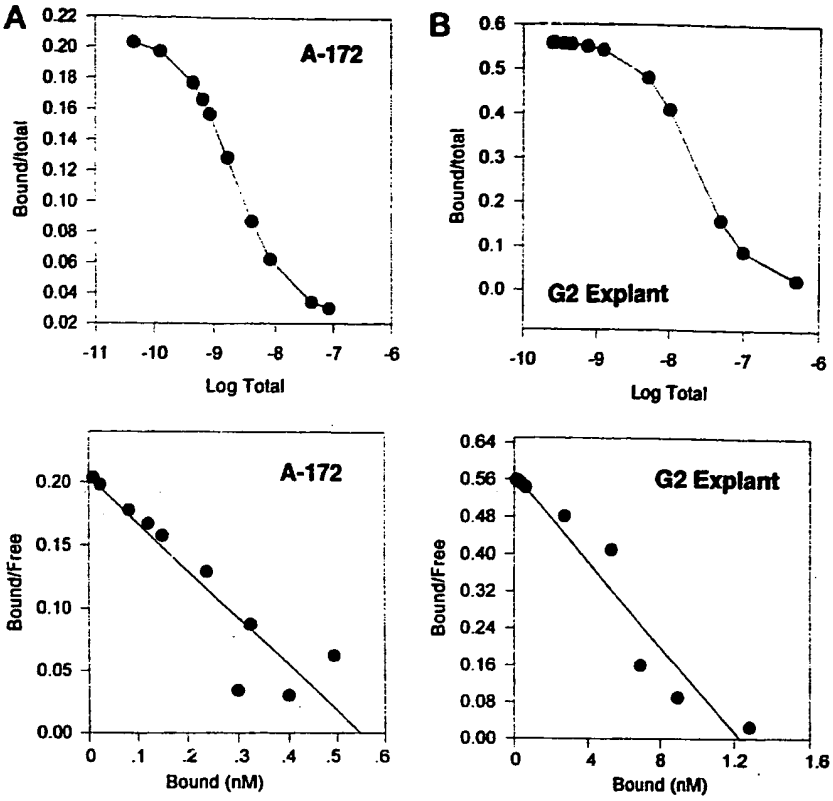

FIG. 5. **Competitive binding assay on A-172 glioblastoma cells (*A*) and human glioma G2 explant cells (*B*).** Data are expressed as a percentage of total ^{125}I-hIL13 binding to cells (*upper panel*) and as a Scatchard plot (*lower panel*). The points are the average of two determinations. Similar data were obtained on three other established glioma cell lines.

Cells—Despite being competitors for the same binding site on some cancer cells, we have observed differences in hIL13- and hIL4-induced cellular effects (14). Namely protein synthesis was inhibited in A431 epidermoid carcinoma cells in a dose-dependent manner by hIL4, whereas hIL13 had no effect on these cells, even at concentrations as high as 10 μg/ml hIL13 for a 72-h incubation (14). Similarly, hIL13 had no effect on the growth of glioma cells. The U-251 MG, U-373 MG glioma cells (Fig. 4), and G2 explant cells (Fig. 4) were unaffected by the 5-day and/or 1-week treatment with IL13. On all these cells, hIL4 had no activity on their growth either (Fig. 4).[2]

hIL13 Binding Affinity to A-172 and G2 Explant Cells—It was important to determine whether the hIL13Rs on the established glioma cell line, such as A-172 glioma cells, have different or similar binding affinity for hIL13 compared with the hIL13R that is expressed on freshly isolated cells. To investigate this, we performed competitive binding assays. As shown in Fig. 5*A*, unlabeled hIL13 competed for the binding of ^{125}I-hIL13 to A-172 cells efficiently (*upper panel*). The Scatchard plot analysis of displacement experiments (*lower panel*) revealed one single binding site for hIL13 of intermediate affinity, K_d = 1.6 nM. There are 22,600 binding sites for hIL13 on the A-172 cell line. The competition of unlabeled hIL13 for the binding of iodinated ligand (Fig. 5*B*, *upper panel*) and the Scatchard analysis performed on G2 explant cells (Fig. 5*B*, *lower panel*) have shown similar results to that obtained on A-172 cells. However, the number of binding sites on explant cells is 300,000 per cell with K_d of 2.4 nM. In another experiment, the estimate of hIL13 binding sites indicated more than 500,000 binding sites per cell.

Thus, there is no difference in affinity of hIL13 to its receptor whether or not cells are permanently cultured or are derived from the primary culture, although the explant cells seem to be considerably more enriched in hIL13 receptors than the established glioma cell cultures.

hIL4 Does Not Compete for Labeled hIL13 Binding Sites but hIL13 Is a Competitor for ^{125}I-hIL4 Binding Sites on Glioma

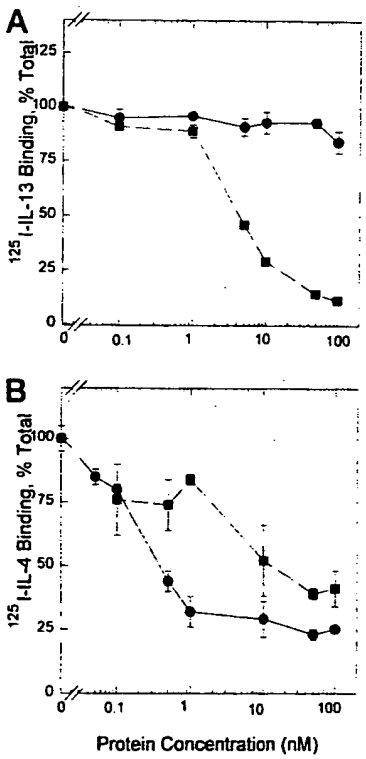

FIG. 6. **Cross-competition between hIL13 and hIL4 for the binding sites of labeled interleukins on glioma cells.** A-172 glioblastoma cells (1×10^6) were incubated with 200 pM ^{125}I-hIL13 (*A*) or ^{125}I-hIL4 (*B*) with or without increasing concentrations (up to 100 nM) of unlabeled hIL13 or hIL4. Bound radioactivity was determined as described under "Experimental Procedures." Data are presented as a mean of % total binding of cells incubated with radiolabeled interleukins only. Total of ^{125}I-hIL13 bound to A-172 cells was 8699 ± 11 (cpm ± S.D.), and total bound ^{125}I-hIL4 was 5789 ± 185 (cpm ± S.D.). The experiments were performed in duplicate. *Bars* represent S.D. when larger than symbol. ●, IL-4; ■, IL-13.

Cells—The first step in a chimeric toxin action is the binding to a specific internalized receptor. Since hIL4 does not neutralize the cytotoxic activity of hIL13-PE38QQR on glioma cells, we performed standard competition experiments at 4 °C using radiolabeled ligands. As seen in Fig. 6*A*. hIL13 displaced labeled hIL13 very efficiently on A-172 glioma cells. However, hIL4 did not compete for the binding of ^{125}I-hIL13 at all at up to 100 nM of the competitor. On the other hand, as shown above (Figs. 2 and 3), hIL13 blocked the cytotoxic actions of both hIL4-PE38QQR and hIL4-PE4E on glioma cells. Therefore, we performed a reverse competition assay and found that either interleukin was a competitor for ^{125}I-hIL4 binding sites (Fig. 6*B*).

Thus, the results of binding experiments suggest that the nonreciprocal interference of interleukins with the cytotoxic activities of their respective chimeric toxins on glioma cells is due to the nonreciprocal interference with the binding to the interleukin receptors.

hIL13 Is Not a Competitor for ^{125}I-hIL4 Binding Sites on Cells Transfected with the 140-kDa hIL4 Receptor—We have shown that hIL13 blocks the cytotoxicities of hIL4-based chimeric toxins and competes for the binding sites of ^{125}I-hIL4 on glioma cells. The 140-kDa hIL4R chain is believed to be a principal hIL4 binding protein. Therefore, we used cells transfected with the hIL4R (CTLL^{hIL4R}; Ref. 15) and performed competition binding assays. We have found that hIL13, unlike hIL4, does not compete for the ^{125}I-hIL4 binding sites on CTLL^{hIL4R} cells (Fig. 7). This result was not unexpected. In similar experiments with the hIL4R transgenes, hIL13 did not compete for labeled hIL4 binding sites (*e.g.* Ref. 25). On the other hand, hIL13 is a competitor for ^{125}I-hIL4 cross-linking to the 140-kDa protein (17, 19, 20). These results and our results obtained on glioma cells using chimeric toxins suggest that the interaction of hIL13 with the hIL4R involves more elements besides the 140-kDa hIL4R chain.

DISCUSSION

We have found that glioma cells exhibit different responses to hIL13- and hIL4-based chimeric proteins containing PE38QQR as well as the two interleukins themselves when compared with adenocarcinoma cells (14). All of the studied glioma cell lines are killed potently by hIL13-PE38QQR, and these killing activities are blocked specifically by an excess of hIL13. On an array of established human glioma cell lines, and represented by the U-373, U-251, DBTRG MG, Hs-683, U-87 MG, SNB-19, and A-172 cell lines, hIL4 cannot block the action of hIL13-based chimeric protein (Table I). The same phenomenon is present on primary cultured human glioma cells. Thus, there is one major form of internalized receptor for hIL13 on glioma cells that does not interact with hIL4. Of interest, a corresponding hIL13-PE38QQR hIL4-based chimeric protein, hIL4-PE38QQR, is weakly active or not active through the specific binding to the hIL4 binding protein (Table I). This is seen on the same cell lines that do respond very well to hIL13-PE38QQR. Thus, the hIL13R in glioma cells is apparently different from the one described previously (Adenocarcinomas in Table I) (14). When hIL4-PE38QQR, or hIL4-PE4E, exerts cytotoxic activity, this activity can be nevertheless neutralized by an excess of hIL13, as seen on adenocarcinoma cell lines (14). These data provide a new insight into the possible mechanisms of interrelatedness between hIL13R and hIL4R in glioma cells.

Studies with a mutated IL4 first suggested interrelatedness between IL13 and IL4 receptors (25). Although the existence of a novel subunit that is shared between the two receptors was postulated, the same group of investigators has recently pointed to an already identified 140-kDa hIL4R chain as the component of the hIL13R (19). This is in support of our previous studies (14, 17) and that of others (20). The model system used in our protocols allows us to employ wild-type interleukins and monitor the effects of hIL4 or hIL13 on the hIL4R- and hIL13R-mediated cellular events. In such a model, we were able to show reciprocal inhibition of the cytotoxic activities of hIL4- and hIL13-based chimeric toxins by the interleukins alone (14). We suggested that in order to explain this phenomenon, the common form of hIL13 and hIL4 receptor on the studied adenocarcinoma cells must be internalized and is composed of a 140-kDa principal subunit of the hIL4R (16) and a 70-kDa hIL13-binding protein (17), which is in agreement with emerging consensus.

Our current data on glioma cells implicate another type of hIL13R that may not involve the 140-kDa subunit of the hIL4R. Several observations speak in favor of such a possibility. First, hIL4-PE38QQR has a very weak activity on most of the glioma cells tested. This result is surprising, since hIL4-PE38QQR tended to be more active from the corresponding hIL13 toxin on several adenocarcinoma cell lines (14). Therefore, glioma cells should express relatively low levels of hIL4 binding sites (as compared with number of hIL13 sites) that, in fact, has already been documented (13). However, even at these levels of hIL4R expression we would expect better cytotoxic activity of hIL4-PE38QQR on these cells (Table I). Second, we

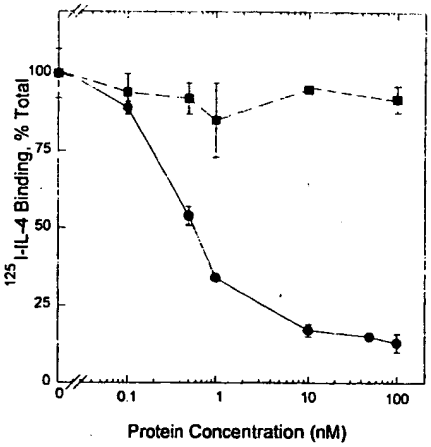

FIG. 7. **Competition of hIL13 for the binding sites of labeled hIL4 on CTLL-2 cells transfected with the human 140-kDa IL4 receptor (CTLL-2^{hIL4R}).** CTLL-2^{hIL4R} were incubated with 200 pM of ^{125}I-hIL4 with or without excess hIL13 or hIL4. The results are expressed as % of total binding. Total ^{125}I-hIL4 bound to cells was 4412 ± 344 (cpm ± S.D.). The experiments were done in duplicate and *bars* represent S.D. when larger than symbols. ●, IL-4; ■, IL-13.

TABLE I

Cytotoxic activities of hIL13- and hIL4-based chimeric toxins and inhibitory potencies of hIL13 and hIL4 to block these activities on cancer cells

	hIL13-PE38QQR, cytotoxicity			hIL4-PE38QQR, cytotoxicity		
	without ILs	with hIL13	with hIL4	without ILs	with hIL13	with hIL4
Gliomas	++++[a]	–	++++	0/++	–	–
Adenocarcinomas[b]	+++	–	–	+++	–	–

[a] Arbitrary estimate of the cytotoxic potency (+ to ++++); cytotoxicity blocked (–).
[b] Based on data in Ref. 14.

have recently shown the lack of involvement of the 140-kDa chain in a hIL13-evoked growth-inhibitory effect on human renal cell carcinoma cells (28). Third, hIL4 is deprived of any ability to influence the action of hIL13-PE38QQR on glioma cells, including freshly cultured explant cells, at even 1000-fold molar excess over the chimeric toxin. This finding is supported by the data obtained in studies on some renal cell carcinoma cells (17, 27) and suggests altogether the presence of cancer-specific receptor for hIL13. Binding experiments using ^{125}I-hIL13 also have shown the lack of hIL4 competition for the radiolabeled ligand binding sites. However, the hIL4R that is present on U-251 MG cells, for example, interacts with hIL13, since hIL13 blocks the cytotoxicity of hIL4-PE38QQR. hIL13 appears to be a good competitor for ^{125}I-hIL4 binding sites in a competitive binding assay on glioma cells. On the other hand, hIL13 does not compete for ^{125}I-hIL4 binding sites on cells transfected with the 140-kDa hIL4R alone. This suggests that the expression of 140-kDa hIL4R is necessary but not sufficient for the interaction with hIL13. The specific molecular forms of this and other hIL13 receptor forms are currently being revealed.[3] One common feature of all these forms is their ability to undergo internalization readily upon binding a ligand, as evidenced by the high effectiveness of hIL13 toxin on various cancer cell lines and explant cells (14, 18).[3]

Established human glioma cells express up to 30,000 binding sites for hIL13 per cell and the explant cells even 10 times more. These binding sites represent a new attractive target for the treatment of brain cancers (18). Since human glioma established cell lines and also human glioma explant cells express an IL4-independent hIL13R, it may be possible to take advantage of this phenomenon pharmacologically.

Acknowledgments—We thank Immunex Corp., Seattle, WA, for providing transfectant cells with the hIL4R transgene.

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[3] N. I. Obiri, P. LeLand, T. Murata, W. Debinski, and R. K. Puri, unpublished results.

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Oppenheimer & Co Inc (1807), USA
SO BioCentury Extra, 25 SEP 1996, vol. 465, Page(s) 1.
BioWorld Today, 25 SEP 1996, vol. 7187, Page(s) 1,5.
TC (General information not published in print edition)
AB Guilford Pharmaceuticals has received Food and Drug Administration (FDA) marketing approval for its Gliadel Wafer biodegradable polyanhydride wafer containing the chemotherapeutic drug, carmustine, for implant into the brain of patients with **glioblastoma multiforme**, a common form of **brain** cancer. The company's share price increased by $1.625 to close at $29.25 following the news.